Identification of a novel *MAP3K1* variant in a family with 46, XY DSD and partial growth hormone deficiency

YIPING CHENG[1-4], CHAO XU[1-4], JIANGFEI YANG[5], XINLI ZHOU[1-4] and NAN CHEN[6]

[1]Department of Endocrinology and Metabolism, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University; [2]Department of Endocrinology and Metabolism, Shandong Provincial Hospital Affiliated to Shandong First Medical University; [3]Institute of Endocrinology, Shandong Academy of Clinical Medicine; [4]Shandong Clinical Medical Center of Endocrinology and Metabolism; Departments of [5]Radiology and [6]Dermatology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong 250021, P.R. China

Received July 27, 2021; Accepted March 15, 2022

DOI: 10.3892/mmr.2022.12854

Abstract. The 46, XY disorder of sex development (DSD) is the main cause of birth defects; however, as it is a group of highly heterogeneous diseases, >50% of cases are not accurately diagnosed. Identification of more cases will improve understanding of the relationship between genotype and phenotype for DSD. The present study conducted a systematic analysis of the clinical characteristics of a proband with 46, XY DSD, applied genetic analysis by whole-exome sequencing to this pedigree and performed bioinformatics analysis of the identified variant. The proband presented with a short penis, lack of testicles and partial growth hormone (GH) deficiency at 1 year old. Histopathological examination revealed there were oviduct, epididymis and fibrous vascular tissue on both sides of the abdomen. The last follow-up at 5 years of age revealed that the patient exhibited restricted growth, a 1.5-cm penis and lack of testicles. Notably, a novel pathogenic mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*) variant (c.3020A>G) was identified in the proband, resulting in a change in the 1,007th amino acid (glutamine) of the encoded protein. This variant caused the uncharged neutral glutamine to be replaced by a positively charged basic arginine. p.Gln1007 in MAP3K1 was confirmed to be conserved across various species. Pathogenicity analysis using bioinformatics tools suggested that this *MAP3K1* variant may cause functional defects. In conclusion, the present study identified a novel *MAP3K1* variant that was the cause of 46, XY DSD and partial GH deficiency. The present findings extend the mutation spectrum of *MAP3K1* and provide novel characteristics of 46, XY DSD.

Correspondence to: Professor Nan Chen, Department of Dermatology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, 324 Jing 5 Road, Jinan, Shandong 250021, P.R. China
E-mail: rhythm_two@sina.com

Abbreviations: DSD, disorder of sex development; SRY, sex-determining region Y; SOX9, SRY-related HMG-box gene 9; MAP3K1, mitogen-activated protein kinase kinase kinase 1; MR, magnetic resonance; GnomAD, Genome Aggregation Database; TOPMED, Trans-Omics for Precision Medicine; ExAC, Exome Aggregation Consortium; HGMD, Human Gene Mutation Database; PCR, polymerase chain reaction; ACMG, American College of Medical Genetics and Genomics; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; AMH, anti-Müllerian hormone

Key words: 46, XY DSD, *MAP3K1*, diagnosis, heterogeneity

Introduction

The 46, XY disorder of sex development (DSD) is defined as the abnormal sexual differentiation process of 46, XY males, with patients presenting with gonads and/or phenotypic sex that is inconsistent with a male genotype (1). Patients with 46, XY DSD are usually born with birth defects and are at a high risk of gonadal tumors, which not only increases physical and mental harm to patients, but also subsequently burdens the family. Notably, an early diagnosis is important for a positive outcome for patients with 46, XY DSD (2). However, the clinical phenotype of 46, XY DSD is highly heterogeneous. The abnormal external genitalia of patients with 46, XY DSD can be manifested as completely male or female; however, more commonly, a phenotype between male and female is presented, with characteristics including hypospadias, cryptorchidism, clitoral enlargement and labia majora fusion (3). Clinical diagnosis of 46, XY DSD is difficult and >50% of cases are not accurately diagnosed (4).

The most frequent causes of 46, XY DSD are androgen insensitivity and gonadal dysgenesis (5), which may involve mutations in numerous genes, including sex-determining region Y (*SRY*), androgen receptor (*AR*), SRY-related HMG-box gene 9 (*SOX9*), steroid 5 α-reductase 2, nuclear receptor subfamily 5 group A member 1 and mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*) (6). Among them, *MAP3K1* (NM_005921.1) variants are a common cause and account for 15-20% of 46, XY DSD [Online Mendelian Inheritance in Man (OMIM) 400044] cases (7). These variants may lead to protein function acquisition that changes co-factor binding and increases phosphorylation of targets in the downstream

MAPK pathway, which can lead to an imbalance between SOX9/FGF9 and WNT/β-catenin signaling and abnormal human sex determination (8). Although these findings strongly suggest that *MAP3K1* variants are involved in the etiology of testicular dysgenesis, it is still necessary to better understand the mechanism underlying the effects of the MAPK pathway on the gene regulatory network of human testicular development (6,9). Therefore, identification of more cases will help to better understand the relationship between genotype and phenotype in DSD caused by *MAP3K1* mutations.

The present study detected a novel pathogenic *MAP3K1* variant (c.3020A>G) in a Chinese patient with 46, XY DSD and partial growth hormone (GH) deficiency. The variant was revealed to be pathogenic and p.Gln1007 in MAP3K1 was shown to be conserved across various species in bioinformatics analysis. The present research expands the mutation spectrum of *MAP3K1* and may be conducive to improved genetic diagnosis and counseling in the future.

Subjects and methods

Patients. The proband was a premature infant who was delivered by caesarean section at 29 weeks' gestation with no testicles detected at birth. At the age of 1 year, the parents of the proband came to Shandong Provincial Hospital (Shandong, China) to receive a diagnosis. The non-consanguineous parents of the patient had a normal phenotype and denied a family history of genetic diseases. All family members received careful clinical examinations and peripheral blood samples were obtained for genetic analyses. The hormone levels of the patient were detected; ultrasonography and pituitary magnetic resonance (MR), histopathological examination and laparoscopy were applied to evaluate the patient's genital development and pituitary development, respectively. The Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University approved the present study (approval no. 2019-147) and written informed consent was obtained from the parents of the patient. The GH stimulation was conducted as follows: The patient fasted for 8 h; on the first day, the patient received an injection of arginine hydrochloride (0.5 g/kg); before arginine injection, and 30, 60 and 90 min after injection, blood was collected to measure GH and blood pressure was monitored. On the third day, oral levodopa (10 mg/kg) was administered, and the same method of blood collection as mentioned for the first day was performed, and blood pressure was monitored.

Clinical follow-up. The patient was followed up by regular visits to determine their specific characteristics and prognosis. In particular, their intellectual and urogenital development, treatment options and potential complications, such as gender anxiety, psychological and cognitive impairment, and the presence of gonadal tumors, were followed up on over 4 years.

Whole-exome sequencing (WES) and Sanger sequencing. Using QIAamp DNA Mini kit (Qiagen GmbH), genomic DNA was isolated from peripheral blood leukocytes for genetic analysis of the pedigree (II-1, II-2 and III-1). Subsequently, DNA from peripheral blood was used for WES; the genes associated with GH deficiency were also studied by WES. Genomic DNA fragmentation, paired-end adaptor ligation, amplification and purification were performed, and human exons were then captured using SeqCap EZ Med Exome Enrichment Kit (Roche Diagnostics). Following post-capture amplification and purification, a DNA library was generated and sequenced using the Illumina HiSeq sequencing platform (Illumina, Inc.). To obtain the coverage and mean read depth of target regions, sequence data alignment to the human reference genome (hg19) and variant calling were performed using NextGene V2.3.4 software (SofGenetics). The average coverage of the exome was >100x, which allowed a deep enough examination of the target region to accurately match >99% of the target exons. Mutations with low coverage in the target area were screened and filtered to ensure the accuracy of data analysis. The quality/integrity of the processed samples were verified by QuBit4 Fluorometer (Thermo Fisher Scientific, Inc.) and Nanodrop one (Thermo Fisher Scientific, Inc.); the type of sequencing nucleotide length was 300 bp (PE150) and the direction of sequencing was paired end; the catalogue number of the sequencing kit was NovaSeq 6000 S4 Reagent kit v1.5 (300 cycles; cat. no. 20028312; Illumina Inc.). The loading concentration of the final library were ~10-20 pM for DNA sequencing.

In addition, the frequency of normal populations (whether there are variants included in the normal population database) were obtained by NextGene V2.3.4 and our in-house scripts from data from Genome Aggregation Database (GnomAD, https://gnomad.broadinstitute.org/), Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org), Trans-Omics for Precision Medicine (TOPMED, https://topmed.nhlbi.nih.gov/), Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), Clinvar and OMIM databases (http://www.omim.org).

A variant was identified as a mutation when it was not found in 500 Chinese controls with data from sequencing companies in dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and in the exome variant server (https://evs.gs.washington.edu/EVS/), or when the allele frequency was <0.001 in the aforementioned databases. According to Standards and Guidelines for the Interpretation of Sequence Variants published by the American College of Medical Genetics and Genomics (ACMG) in 2015 (10), pathogenic variants were determined with Human Genome Variation Society nomenclature.

Candidate variants were detected by WES. Subsequently, detected pathogenic or suspected pathogenic variants were verified by Sanger sequencing. The following tagged sequencing primers were designed for *MAP3K1*: Forward primer, 5'-CAACAACAACAACAACAGAG-3'; reverse primer, 5'-TGAGGAGATGCAGAAGGT-3. Polymerase chain reaction (PCR) was carried out in a 50-μl reaction system including 5 μl 10X PCR buffer, 4 μl genomic DNA obtained as aforementioned, 4 μl dNTPs, 1 μl forward and reverse primers, and 0.3 μl Taq Hot Start (all from Takara Bio, Inc.). The PCR conditions consisted of an initial denaturation step (95°C for 5 min), followed by 40 cycles of denaturation (95°C, 30 sec), annealing (65°C, 30 sec) and elongation (72°C, 30 sec). Using an ABI 3730 system (Applied Biosystems; Thermo Fisher Scientific, Inc.), amplicons were sequenced. Sequence analysis was performed using the autoassembler software Chromas 2.6.6 (Technelysium Pty, Ltd.).

Bioinformatics analysis. To confirm the conservation of amino acids at mutated positions, Clustal W (UCD) software (https://www.ebi.ac.uk/Tools/msa/clustalo/) was applied to compare 20 species. In addition, online software, such as Mutation Taster (https://www.mutationtaster.org/), PolyPhen v.2 (http://genetics.bwh.harvard.edu/pph2/) and PROVEAN (http://provean.jcvi.org/index.php), was used to predict the potential pathogenic effects of the variants. The Humvar model in PolyPhen v.2 is adapted to assess variations associated with Mendelian genetic diseases and can distinguish severely harmful variations from other human variants. The HumDiv model is suitable for evaluating rare variants that may be involved in complex diseases or in high-density regions of GWAS studies. Therefore, this study is more suitable for Humvar model.

Using I-TASSER software (https://zhanglab.ccmb.med.umich.edu/I-TASSER/), modeling of wild type and mutant proteins was performed. PyMOL Viewer 2.0 (http://pymol.sourceforge.net). was employed to observe the effect of the variants on the protein.

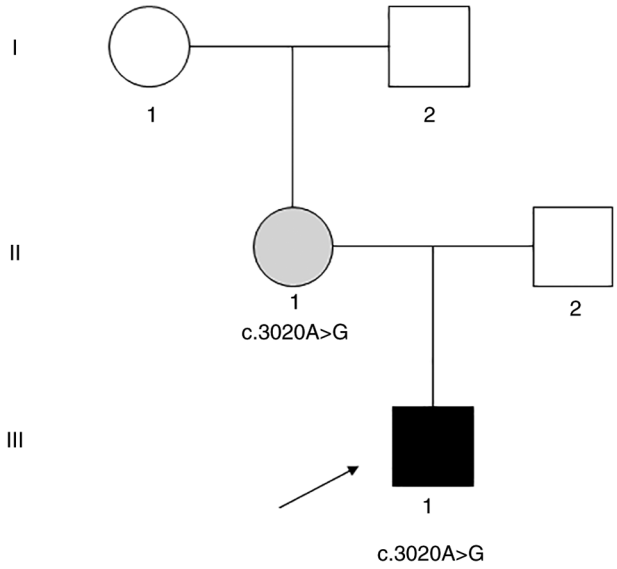

Figure 1. Pedigree including the proband. The arrow and black squares indicate that the proband has 46, XY disorder of sex development. The grey circle indicates that the individual carries the mutant gene but has a healthy phenotype. Circles indicate female individuals; squares indicate male individuals.

Results

Clinical manifestation. Due to reduced fetal movement during pregnancy, the proband of the present study was delivered by cesarean section at 29 weeks' gestation with a birth weight of 1.26 kg. The newborn classification was low birth weight and small for gestational age. After birth, the infant was found to have a short penis, low muscle tension and no testicles. Growth and development lagged behind those of their peers; however, the proband's intellectual development was basically normal. The lineage of the patient is shown in Fig. 1.

The patient was assessed at our hospital at the age of 1 year. Physical examination showed a small penis, lack of bilateral testicles, slightly reduced muscle tension in the limbs and short stature. Their height was 65.7 cm (target height, 76.5±2.6 cm) and body weight was 8.9 kg (target weight, 10.05±1.05 kg); in addition, their parents' height and body weight were relatively low (father: height, 176 cm and weight, 65 kg; mother: height, 159 cm and weight, 54 kg). Bone age test showed an age of 6 months. According to an abdominal ultrasound, the patient presented with bilateral scrotum emptiness, and no testicular tissue was detected in groin and pelvic regions.

The karyotype analysis revealed 46, XY in 50 metaphases. The estradiol, inhibin B and insulin-like growth factor 1 levels were low for a child, whereas the luteinizing hormone (LH), follicle-stimulating hormone (FSH) and cortisol levels were high. Furthermore, testosterone, prolactin and adrenocorticotropic hormone levels were normal for a child (Table I).

GH stimulation test indicated that in response to stimuli, a low level of GH was detected, and the presence of partial GH deficiency was suggested. The gonadotropin-releasing hormone excitation test showed that the patient had a normal pituitary response (Table II). Pituitary MR examination revealed that the upper edge of the pituitary gland was raised.

Follow up. The patient underwent laparoscopy at the age of 2.0 years and no testes were detected in the abdominal cavity and inguinal region. Their height was 80.2 cm (target height, 88.5±3.4 cm) and body weight was 11.40 kg (target weight, 12.54±1.23 kg). Histopathological examination revealed there were oviduct, epididymis and fibrous vascular tissue on both sides of the abdomen, but no obvious convoluted tubule and follicular tissue. Hormone measurements at 2.0 years indicated low estradiol AMH and inhibin B-levels for a child, whereas the levels of LH and FSH were high. Testosterone levels was decreased from year 1-year 2 (Table I).

The patient identified as male at the last follow-up when they were 5 years of age. Their height was 110.2 cm (target height, 111.3±4.4 cm) and body weight was 16.5 kg (target weight, 18.98±1.97 kg). Gynecological examination confirmed male external sexual organs with a lack of testicles. The length of the penis was 1.5 cm.

The mental development of the patient was normal. During the follow-up period, no medication was used, and psychological or cognitive impairment, gender anxiety disorder and gonadal tumors were not observed.

Mutation detection. The WES technique was applied to the genetic analysis of the pedigree to further search for the disease-causing genes. Notably, a novel heterozygous *MAP3K1* variant (c.3020A>G) was identified in the patient and their mother, which resulted in a change in the 1,007th amino acid of the encoded protein from glutamine to arginase (Gln1007Arg; Fig. 2A). Since no substitution was detected in TOPMED, GnomAD and ExAC, this variant was not considered polymorphic. The C-terminus of MAP3K1 has a serine/threonine kinase domain; upstream of this domain is a conserved caspase-3 cleavage site and a ubiquitin interaction motif. Two zinc finger structures, including one RING structure and one SWIM region, are located at the N-terminus of MAP3K1 (11). The heterozygous variant was located at position 1,007 (Gln1007Arg), upstream of the serine/threonine kinase domain (Fig. 2B). PolyPhen v.2 predicted the hemizygous *MAP3K1* variant to be potentially damaging with a score of 0.953 (Fig. 2C), and this variant was predicted to be

Table I. Hormone measurements of the patient.

Patient age	LH, mIU/l	FSH, mIU/l	T, ng/ml	E, pg/ml	AMH, ng/ml	ACTH, pg/ml	COR, nmol/l	PRL, ng/ml	IGF-1, ng/ml	Inhibin B, pg/ml	TSH, mIU/ml	FT3, pmol/l	FT4, pmol/l
1.0 year	20.76	160.51	0.78	1.54	0.01	33.09	503.90	11.21	<25.0	<10.0	2.981	6.1	14.21
2.0 year	35.68	141.59	0.11	1.68	1.20	-	-	-	-	10.0	-	-	-

AMH, anti-Müllerian hormone; ACTH, adrenocorticotropic hormone; COR, cortisol; E, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; T, testosterone; IGF-1, insulin-like growth factor 1; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine. Normal reference values for the patient's age: LH, 1.0-11.4 mIU/l; FSH, 1.7-7.7 mIU/l; T, 0.06-0.82 ng/ml; E, 22.3-341 pg/ml; AMH, 1.66-9.49 ng/ml; ACTH, 7.2-63.3 pg/ml; COR, 172-497 nmol/l; PRL, 4.79-23.3 ng/ml; IGF-1, 55-327 ng/ml; inhibin B, 21-166 pg/ml; TSH, 0.64-6.27 mIU/ml; FT3, 3.5-6.5 pmol/l; FT4, 11.5-22.7 pmol/l.

Table II. Results of GH stimulation test and gonadotropin-releasing hormone excitation test of the patient.

Hormone	0 min	30 min	60 min	90 min
GH, ng/ml	1.70	2.04	5.01	3.82
FSH, mIU/l	160.51	>205.0	>205.0	>205.0
LH, mIU/l	20.76	105.52	137.03	140.46

GH, growth hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

neutral by PROVEAN with a score of -0.557. Mutation Taster showed that the pathogenic variant could influence protein properties. According to ACMG criteria, the *MAP3K1* variant had 'unknown significance'. Using the Clustal W tool, multiple amino acid sequence alignments suggested that p.Gln1007 is conserved across various species (Fig. 3). Currently, the 3D MAP3K1 protein cannot be crystalized; therefore, structural analysis could not be performed. In addition, there were no other mutations associated with hypogonadism in the exome of the patient. Therefore, it was hypothesized that this rare *MAP3K1* mutation (c.3020A>G) may be associated with the occurrence of 46, XY DSD. The presence of allelic variants in the main genes associated with GH deficiency were excluded.

Discussion

The present study identified a novel heterozygous *MAP3K1* variant (c.3020A>G, p.Gln1007Arg) in a patient with 46, XY DSD. The GH levels of the patient were evaluated and a partial GH deficiency was detected. Follow-up for 4 years indicated that the patient had a good prognosis except for developmental delay. The present findings enrich the *MAP3K1* variant spectrum and contribute to the study of genotype-phenotype relationships in patients with 46, XY DSD.

The expression of the *SRY* gene (MIM 480000) on the Y chromosome controls male sex determination, leading to the development of undifferentiated gonads into testes (12,13). Sertoli cells in the testis produce anti-Müllerian hormone, which causes the degradation of Müllerian ducts, whereas Leydig cells secrete testosterone to promote Wolffian duct differentiation into seminal vesicles, vas deferens and epididymis. In the absence of the Y chromosome and *SRY* expression, undifferentiated gonads develop into ovaries, Müllerian ducts form the uterus, fallopian tubes and the upper third of the vagina, and Wolffian ducts degenerate. This process occurs approximately from the 4th week to the 12th week of pregnancy, and is performed in a strictly timed and gene dose-dependent manner (12). If there is a problem with gonad development, it can lead to DSDs (14,15). Notably, 46, XY DSD may be caused by functional mutations in genes (16,17) and a missense mutation of the *MAP3K1* gene is a common cause of 46, XY DSD (18). In the present study, a missense mutation, c.3020A>G, was found in exon 14, which changed the 1,007th amino acid of the encoded protein from glutamine to arginase; glutamine is an uncharged neutral amino acid in wild type MAP3K1, which in this mutation is replaced by the

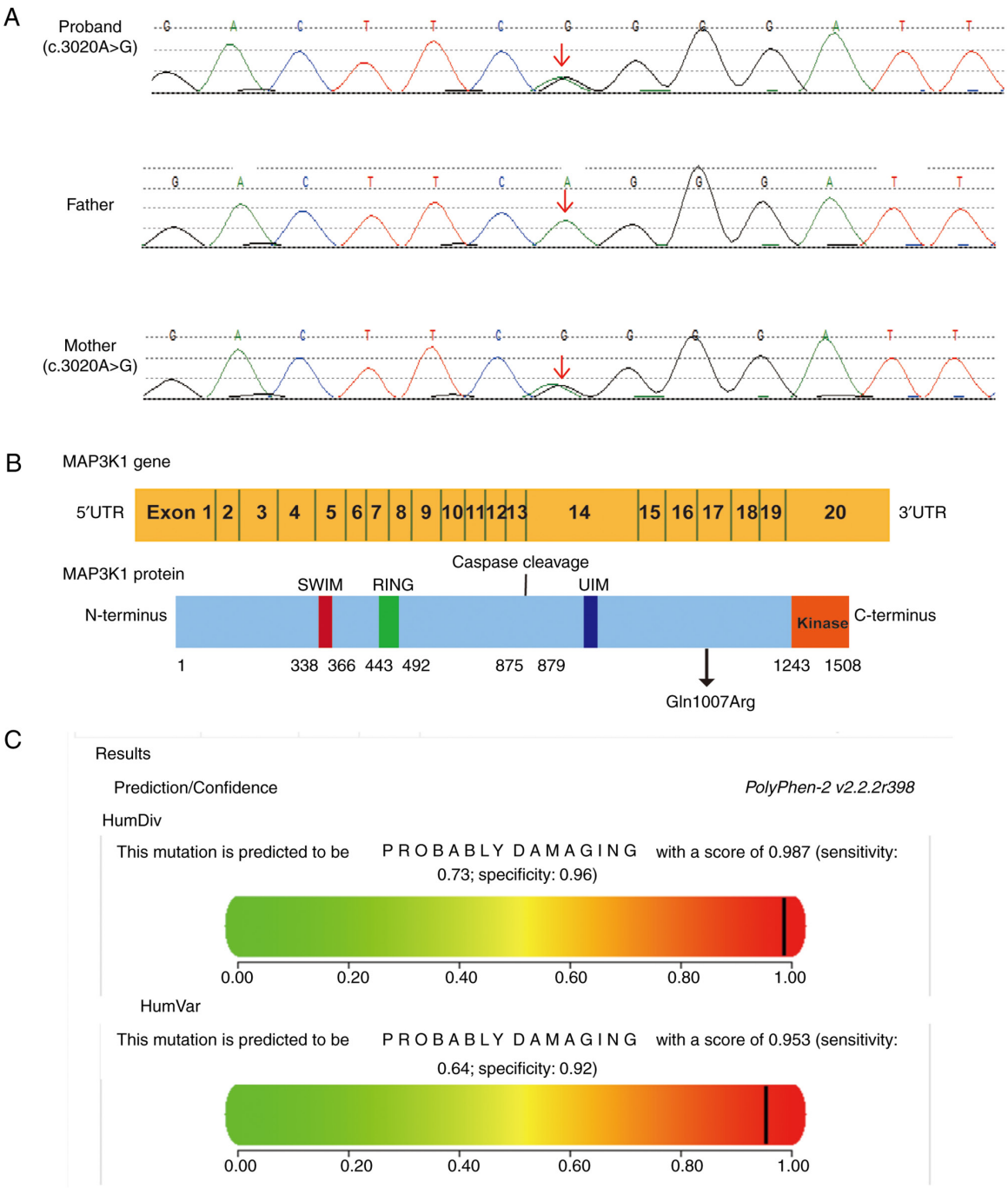

Figure 2. Sequencing results and bioinformatics analysis of the *MAP3K1* gene variant. (A) Partial sequence diagram of *MAP3K1*. The arrow shows a rare heterozygous variant in *MAP3K1* (c.3020A>G) in I-2 and II-1 individuals, resulting in the 1,007th amino acid of the encoded protein being changed from glutamine to arginase. (B) Domains of the MAP3K1 proteins. Below the domains, variants at the protein level are shown. (C) Prediction of the rare damaging variant c. 3020A>G (p.Gln1007Arg) in Polyphen v.2. MAP3K1, mitogen-activated protein kinase kinase kinase 1.

positively charged basic amino acid arginine. The secondary structure of the protein may be affected by this change in charge. The conservation of the p.Gln1007 residue and a high pathogenic probability of this *MAP3K1* mutation indicates the importance of the residue in the structure and function of the protein. Similarly, Das *et al* (19) identified a missense mutation in exon 14 of the *MAP3K1* gene (c.2416G>A, p.Asp806Asn) in four patients with hypospadias with gonadal abnormalities, which resulted in aspartic acid at position 806 of the amino acid sequence being replaced by asparagine; computer analysis indicated this missense mutation was a pathogenic mutation.

To date, numerous mutations have been identified in the open reading frame or non-coding region of the *MAP3K1* gene from the HGMD database; however, only 15 missense mutations of the *MAP3K1* gene have been reported to cause 46, XY DSD (Table III). Pearlman *et al* (20) identified three missense mutations of *MAP3K1* in a family from New Zealand, which included five women with complete gonadal dysfunction (c.1846G>A, p.Gly616Arg) and two patients with sporadic 46, XY gonadal dysgenesis (c.566T>C, p.Leu189Pro and c.566T>G, p.Leu189Arg). PolyPhen and SIFT revealed that p.Leu189Pro and p.Leu189Arg mutations

Table III. Reported missense mutations of the *MAP3K1* gene in 46, XY DSD.

First author, year	Coding change	Protein change	Zygosity	Exon	Phenotype	Functional study	(Refs.)
Eggers, 2016	G → C	p.Asp132His	Heterozygous	1	DSD	No	(22)
Loke, 2014	C → T	p.Pro153Leu	Heterozygous	1	GD	Yes	(21)
Pearlman, 2010;	T → C	p.Leu189Pro	Heterozygous	2	CGD	Yes	(20)
Loke, 2014;							(21)
Granados, 2017							(23)
Pearlman, 2010;	T → G	p.Leu189Arg	Heterozygous	2	CGD	Yes	(20)
Loke, 2014							(21)
Granados, 2017	T→ A	p.Leu189Gln	Heterozygous	2	CGD	No	(23)
Eggers, 2016	A → G	p.Gln237Arg	Heterozygous	3	46, XY DSD	No	(22)
Baxter, 2015	C→ T	p.Pro257Leu	Heterozygous	3	DSD	No	(16)
Eggers, 2016	A → T	p.Met312Leu	Heterozygous	4	46, XY DSD	No	(22)
Baxter, 2015	G → A	p.Arg339Gln	Heterozygous	4	CGD	No	(16)
Granados, 2017	T → A	p.Leu587His	Heterozygous	10	CGD	No	(23)
Pearlman, 2010;	G → A	p.Gly616Arg	Heterozygous	10	CGD	No	(20)
Baxter, 2015							(16)
Eggers, 2016	T → C	p.Cys691Arg	Heterozygous	11	CGD	No	(22)
Xue, 2019	T → G	p.Leu706Arg	Heterozygous	12	CGD	Yes	(24)
Granados, 2017	T → G	p.Leu764Arg	Heterozygous	13	PGD	No	(23)
Das, 2013	G → A	p.Asp806Asn	Heterozygous	14	46, XY DSD	No	(19)
The present study	A → G	p.Gln1007Arg	Heterozygous	14	46, XY DSD	No	-
Eggers, 2016	C → T	p.Ala1443Val	Heterozygous	19	46, XY DSD	No	(22)

DSD, disorder of sex development; GD, gonadal dysgenesis; CGD, complete gonadal dysgenesis; PGD, partial gonadal dysgenesis.

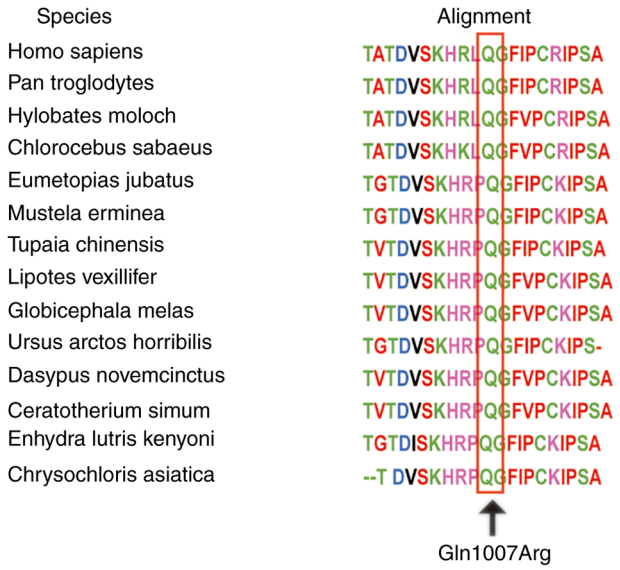

Figure 3. Cross-species conservation of mitogen-activated protein kinase kinase kinase 1 p.Gln1007.

may have interference functions, whereas p.Gly616Arg mutations did not; however, this mutation caused the conservative neutral amino acid at this position to be replaced by a basic amino acid. Further experiments revealed that the missense mutations identified in the two sporadic cases changed the phosphorylation of downstream targets p38 and ERK1/2, and enhanced the binding of RHOA to the MAP3K1 complex; the findings of Loke *et al* (21) also identified a p.Pro153Leu variant in a patient with gonadal dysgenesis. Baxter *et al* (16) reported on two cases that had the p.Gly616Arg variant, of which one was a female with complete gonadal dysgenesis and another was a male with ovotesticular DSD; furthermore, a patient with complete gonadal dysgenesis had a *de novo* p.Arg339Gln missense variant and a male patient with complex ambiguous genitalia but without gonad hypoplasia had a p.Pro257Leu missense variant. Eggers *et al* (22) inferred 10% prevalence of *MAP3K1* variants amongst patients with 46, XY DSD in a smaller cohort and this could be up to 18% if the *MAP3K1* phenotypic spectrum was expanded. In addition, two *MAP3K1* variants (p.Met312Leu and p.Ala1443Val) were identified in multiple patients with a variety of phenotypes, including complete or partial gonadal dysgenesis, hypospadias and under-virilization. Granados *et al* (23) performed clinical evaluation, endocrine evaluation and genetic analysis of six individuals with 46, XY DSD from four unrelated families. Three of these patients exhibited complete gonadal dysgenesis with *MAP3K1* variants (c.1760T>A, p.Leu587His; c.2291T>G, p.Leu764Arg; c.566T>A, p.Leu189Gln). In addition, Xue *et al* (24) detected a missense mutation (c. 2117T>G; p.Leu706Arg) in exon 12 of the *MAP3K1* gene in a patient with 46, XY DSD and a missense mutation (c.2416G>A; p.Asp806Asn) was identified in exon 14 of the *MAP3K1* gene in patients with 46, XY DSD by Das *et al* (19). Notably, Pearlman *et al* (20) found only one

of these individuals in pedigrees from two families of interest exhibiting sex-limited autosomal-dominant mendelian inheritance of 46, XY DSD had micropenis and cryptorchidism (III-13). The absence of hypospadias was found in the proband of the present study, which is different from complete hypogonadism. The missense mutation of *MAP3K1* is a common cause of 46, XY DSD. It was speculated that the location of missense mutations may affect the function of genes or encoded proteins, leading to differences in clinical phenotypes of patients. Alternatively, interactions between this gene and other genes may also be responsible for this phenomenon, but further research is needed.

It is worth noting that in addition to 46, XY DSD, the present proband exhibited partial GH deficiency. GH deficiency is the most common type of restricted growth and can present as a simple GH deficiency or with multiple pituitary hormone deficiencies. Previous studies have reported that genetic mutations are an important cause of idiopathic GH deficiency. Currently known pathogenic genes of GH deficiency include *GH* gene, GH-releasing hormone receptor gene, and *POU1F1* gene (25,26). To date, no patients with 46, XY DSD have been reported to have GH deficiency and no research has identified a causal relationship between *MAP3K1* and GH deficiency; this needs to be further explored in functional tests.

In the gene network responsible for gonadal development, *MAP3K1* is an important component. *MAP3K1* is involved in the regulation of the transcription of so many important genes, and serves a key role in cell proliferation, differentiation and apoptosis (27,28). Studies have revealed that variants in the *MAP3K1* gene can lead to changes in cofactor binding and increase the phosphorylation of targets in the downstream MAPK pathway, including ERK1/2 and p38, ultimately leading to the occurrence of 46, XY DSD. Increased phosphorylation of ERK1/2 may lead to decreased expression of *SOX9*, whereas increased phosphorylation of p38 may increase the expression of *CTNNB1*, which are important signaling molecules in testicular or ovarian-promoting pathways (19,21,29-31). Chamberlin *et al* (29) showed that mutations in the *MAP3K1* gene have different effects on protein binding, depending on the domain in which the mutation occurs. Generally, the net effect of increased binding of the pro-kinase RHOA and MAP3K4 proteins, and decreased binding of the inhibitory RAC1 protein results in the observed gain of function with *MAP3K1* mutations. GH signaling is necessary for GH to function. Firstly, GH is combined with GH receptor (GHR) to establish the post-GHR process, and the most important GHR-mediated signaling pathways include GHR/JAK2/SHC/MAPK, GHR/JAK2/STATs and GH/IRS/PI3K/Akt pathways (32). Notably, Jin *et al* (33) demonstrated that JAK2 was required for ERK signaling in response to GH in preadipocytes and hepatoma cells. Therefore, it is reasonable to speculate that the *MAP3K1* variant identified in the patient assessed in the present study may cause changes in GHR-mediated signaling pathways and ultimately lead to the occurrence of partial GH deficiency.

The previously reported genetic pattern of pathogenic *MAP3K1* variants is autosomal dominant and sex-limited (20,23,24). In two large families and two of 11 sporadic cases with 46, XY DSD, *MAP3K1* variants were first reported with an autosomal dominant, sex-limited pattern of transmission (20). Phenotypic manifestations of the large family reported a range from hypospadias and cryptorchidism to complete hypogonadism; however, there were eight and four unaffected female carriers, respectively. Granados *et al* (23) reported that six individuals from four unrelated families with pathogenic *MAP3K1* variants had both partial and complete gonadal dysgenesis. In addition, 46, XX females carrying *MAP3K1* variants were not affected. Furthermore, Xue *et al* (24) speculated that the unaffected mother of a patient with 46, XY DSD may carry the same *MAP3K1* mutation; however, the mother was not genetically tested. The present findings are consistent with the aforementioned studies; the mother of the proband had the same variant as the proband, but remained phenotypically healthy. These findings indicated that the genetic pattern of the novel pathogenic *MAP3K1* variant (c.3020A>G, p.Gln1007Arg) may be based on autosomal dominant and sex-restricted patterns.

The present study has some limitations. The patient was diagnosed with 46, XY DSD based on clinical data, imaging characteristics and genetic testing results; nevertheless, the association between the phenotype and genotype of 46, XY DSD is not known, and more patients need to be studied to supply more comprehensive information. In addition, more functional tests are needed to verify the findings of the present study.

In conclusion, the present study detected a novel pathogenic *MAP3K1* variant (c.3020A>G) in a patient with 46, XY DSD and partial GH deficiency. The present study expands the mutation spectrum of *MAP3K1*, and will be conducive to improved genetic diagnosis and counseling in the future. WES may find more genetic variants that expound the heterogeneity associated with *MAP3K1* variants.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the National Natural Science Foundation (grant nos. 81670720 and 81974124), special funds for Taishan Scholar Project (grant no. tsqn20161071) and the Shandong Key R&D Program (grant no. 2017GSF18129).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YC, CX and NC designed the present study. YC, JY and XZ performed the present study and analyzed the data. YC wrote the main manuscript. CX and NC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was in line with the Declaration of Helsinki (as revised in Brazil 2013). The Ethics Committee

of Shandong Provincial Hospital affiliated to Shandong University approved this study (approval no. 2019-147), and written informed consent was obtained from the parents of the patient.

Patient consent for publication

Written informed consent was obtained from the parents of the patient.

Competing interests

The authors declare that they have no competing interests.

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